

CLAIMS:

1. A preparative method for isolating RNA comprising an oligo- or polynucleotide from a sample, which method comprises:
  - 5 (a) treating the sample with a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA under conditions so that a proportion of the 2'-OH positions of the ribose rings bear a substituent; and
  - 10 (b) preparing isolated RNA therefrom by separating material containing the substituent from the sample on the basis of a property of the substituent.
2. A method according to claim 1, wherein step (a) is  
15 carried out in a reaction medium which comprises an organic solvent.
3. A method according to claim 2, wherein the organic solvent comprises an organic base.  
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4. A method according to claim 2 or claim 3, wherein the reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole.
- 25 5. A method according to claim 4, wherein the reaction medium further comprises an acylation catalyst.
6. A method according to any one of claims 2 to 5, wherein the reaction medium further comprises water.  
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7. A method according to any one of the preceding claims, wherein the RNA comprises mRNA, rRNA or viral RNA.

8. A method according to any one of the preceding claims, wherein the sample comprises a sample from a biological source.
- 5 9. A method according to any one of the preceding claims, wherein the sample includes DNA.
10. A method according to any one of the preceding claims, wherein the substituent comprises a solid phase.
- 10 11. A method according to claim 10, wherein the solid phase comprises benzoyl chloride polymer bound (BCPB) beads, silica particles or particles of a glass.
- 15 12. A method according to claim 10 or claim 11, wherein the solid phase is modified to introduce a reactive group which reactive group is capable of reacting with RNA to capture the RNA on the solid phase.
- 20 13. A method according to claim 12, wherein the reactive group is introduced by modifying the solid phase with a bi-functional acid halide.
- 25 14. A method according to any one of claims 1-9, wherein the substituent comprises a hydrophobic substituent.
15. A method according to claim 14, wherein the hydrophobic substituent comprises a substituent, OR,
- 30 wherein R comprises C<sub>1</sub>-C<sub>36</sub> alkyl; C<sub>1</sub>-C<sub>36</sub> alkenyl; C<sub>1</sub>-C<sub>36</sub> alkynyl; C<sub>1</sub>-C<sub>36</sub> haloalkyl; C<sub>1</sub>-C<sub>36</sub> aminoalkyl; C<sub>1</sub>-C<sub>36</sub> alkoxyalkyl; C<sub>1</sub>-C<sub>36</sub> alkylthioalkyl; C<sub>1</sub>-C<sub>36</sub> alkoxyalkoxyalkyl; C<sub>1</sub>-C<sub>36</sub> haloalkoxyalkyl; C<sub>1</sub>-C<sub>36</sub> aminoalkoxyalkyl; C<sub>6</sub>-C<sub>36</sub> aryl; C<sub>6</sub>-C<sub>36</sub> alkylaryl; C<sub>6</sub>-C<sub>36</sub> arylalkyl; C<sub>6</sub>-C<sub>36</sub> arylalkenyl; C<sub>1</sub>-C<sub>36</sub> alkanoyl; C<sub>1</sub>-C<sub>36</sub>
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- alkenoyl; C<sub>1</sub>-C<sub>36</sub> haloalkenoyl; C<sub>1</sub>-C<sub>36</sub> haloalkanoyl; C<sub>2</sub>-C<sub>36</sub> haloformylalkanoyl; C<sub>1</sub>-C<sub>36</sub> C<sub>1</sub>-C<sub>36</sub> aminoalkanoyl; C<sub>1</sub>-C<sub>36</sub> azidoalkanoyl; C<sub>1</sub>-C<sub>36</sub> carboxyalkanoyl; C<sub>1</sub>-C<sub>36</sub> carboxyalkenoyl; C<sub>1</sub>-C<sub>36</sub> carboxyalkynoyl; C<sub>1</sub>-C<sub>36</sub> alkylaminoarylalkanoyl; C<sub>1</sub>-C<sub>36</sub> alkoxycarbonyl; C<sub>1</sub>-C<sub>36</sub> alkenyloxycarbonyl; C<sub>1</sub>-C<sub>36</sub> alkylsulfonyl; C<sub>6</sub>-C<sub>36</sub> arylalkanoyl; C<sub>6</sub>-C<sub>36</sub> arylalkenoyl; C<sub>6</sub>-C<sub>36</sub> aryloxyalkanoyl; C<sub>6</sub>-C<sub>36</sub> alkylarylalkanoyl; C<sub>6</sub>-C<sub>36</sub> haloarylalkanoyl; C<sub>6</sub>-C<sub>36</sub> aminoarylalkanoyl; C<sub>1</sub>-C<sub>36</sub> alkylsilanyl; C<sub>1</sub>-C<sub>36</sub> trialkylsilanyl or C<sub>12</sub>-C<sub>28</sub> diarylphosphano; or a substituent R', wherein R' comprises C<sub>1</sub>-C<sub>36</sub> alkyl; C<sub>1</sub>-C<sub>36</sub> alkenyl; C<sub>1</sub>-C<sub>36</sub> alkynyl; C<sub>1</sub>-C<sub>36</sub> haloalkyl; C<sub>1</sub>-C<sub>36</sub> aminoalkyl; halo; amino; C<sub>1</sub>-C<sub>36</sub> alkylamino; C<sub>6</sub>-C<sub>36</sub> aryl; C<sub>1</sub>-C<sub>36</sub> alkylaryl or C<sub>1</sub>-C<sub>36</sub> arylalkyl.
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16. A method according to claim 15, wherein the hydrophobic substituent comprises a C<sub>4</sub> to C<sub>7</sub> carbon chain or ring.
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17. A method according to claim 16, wherein the reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride.
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18. A method according to claim 16 or claim 17, wherein the proportion of 2'-OH positions bearing the substituent is at least 10%.
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19. A method according to claim 15, wherein the hydrophobic substituent comprises a C<sub>8</sub>-C<sub>12</sub> carbon chain or ring.
20. A method according to claim 19, wherein the proportion of 2'-OH positions bearing the substituent is in the range 1 to 10%.

21. A method according to claim 15, wherein the hydrophobic substituent comprises a C<sub>12</sub>-C<sub>36</sub> carbon chain or ring.
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22. A method according to claim 21, wherein the proportion of 2'-OH positions bearing the substituent is up to 1%
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23. A method according to any one of claims 14 to 22, wherein step (b) comprises contacting the treated sample from step (a) with a hydrophobic solid phase so as to bind the material containing the hydrophobic substituent and optionally washing the material bound to the solid phase.
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24. A method according to claim 23, wherein the hydrophobic solid phase comprises hydrophobic particles.
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25. A method according to claim 23 or claim 24, which further comprises a step of eluting the material bound to the hydrophobic solid phase by treating with a detergent, a chaotrope or a solvent, by lowering the salt concentration or by cleaving the substituent from the 2'-OH position of the ribose rings.
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26. A method according to any one of claims 14 to 25, wherein step (b) comprises treating the treated sample from step (a) with a lyotropic salt to aggregate the material containing the hydrophobic substituent as an RNA precipitate, and isolating the precipitate.
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27. A method according to claim 26, wherein the lyotropic salt comprises ammonium sulphate, an alkali

metal chloride, magnesium chloride or calcium chloride.

28. A method according to any one of claims 14 to 22, wherein step (b) comprises treating the treated sample  
5 with a non-polar solvent to form a hydrophobic liquid phase which contains the material containing the hydrophobic substituent, and isolating the hydrophobic liquid phase.

10 29. A method according to claim 28, wherein the non-polar solvent comprises pentane, cyclohexane, toluene, benzene, light petroleum, xylene or hexane.

30. A kit for the preparative isolation of RNA  
15 comprising an oligo- or polynucleotide from a sample, which kit comprises:  
(i) a reaction system for modifying the RNA to form a modified oligo- or poly-nucleotide in which a proportion of the 2'-OH positions of the ribose rings bear a  
20 substituent; and  
(ii) a separation system for preparing isolated RNA by separating material containing the substituent from the sample on the basis of a property of the substituent.

25 31. A kit according to claim 30, wherein the reaction system comprises:

(a) an organic solvent; and  
(b) a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA in the presence  
30 of the organic solvent.

32. A kit according to claim 31, wherein the organic solvent comprises an organic base.

33. A kit according to claim 31 or claim 32, wherein reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole.
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34. A kit according to claim 33, which further comprises an acylation catalyst.
35. A kit according to any of claims 31 to 34, wherein
- 10 the substituent comprises a solid phase.
36. A kit according to claim 35, wherein the solid phase comprises benzoyl chloride polymer bound (BCPB) beads, silica particles or particles of a glass.
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37. A kit according to any one of claims 31 to 34, wherein the substituent comprises a hydrophobic substituent.
- 20 38. A kit according to claim 37, wherein the hydrophobic substituent comprises a substituent, OR, wherein R comprises C<sub>1</sub>-C<sub>36</sub> alkyl; C<sub>1</sub>-C<sub>36</sub> alkenyl; C<sub>1</sub>-C<sub>36</sub> alkynyl; C<sub>1</sub>-C<sub>36</sub> haloalkyl; C<sub>1</sub>-C<sub>36</sub> aminoalkyl; C<sub>1</sub>-C<sub>36</sub> alkoxyalkyl; C<sub>1</sub>-C<sub>36</sub> alkylthioalkyl; C<sub>1</sub>-C<sub>36</sub> alkoxyalkoxyalkyl; C<sub>1</sub>-C<sub>36</sub>
- 25 haloalkoxyalkyl; C<sub>1</sub>-C<sub>36</sub> aminoalkoxyalkyl; C<sub>6</sub>-C<sub>36</sub> aryl; C<sub>6</sub>-C<sub>36</sub> alkylaryl; C<sub>6</sub>-C<sub>36</sub> arylalkyl; C<sub>6</sub>-C<sub>36</sub> arylalkenyl; C<sub>1</sub>-C<sub>36</sub> alkanoyl; C<sub>1</sub>-C<sub>36</sub> alkenoyl; C<sub>1</sub>-C<sub>36</sub> haloalkenoyl; C<sub>1</sub>-C<sub>36</sub> haloalkanoyl; C<sub>2</sub>-C<sub>36</sub> haloformylalkanoyl; C<sub>1</sub>-C<sub>36</sub> C<sub>1</sub>-C<sub>36</sub> aminoalkanoyl; C<sub>1</sub>-C<sub>36</sub> azidoalkanoyl; C<sub>1</sub>-C<sub>36</sub>
- 30 carboxyalkanoyl; C<sub>1</sub>-C<sub>36</sub> carboxyalkenoyl; C<sub>1</sub>-C<sub>36</sub> carboxyalkynoyl; C<sub>1</sub>-C<sub>36</sub> alkylaminoarylalkanoyl; C<sub>1</sub>-C<sub>36</sub> alkoxycarbonyl; C<sub>1</sub>-C<sub>36</sub> alkenyloxycarbonyl; C<sub>1</sub>-C<sub>36</sub> alkylsulfonyl; C<sub>6</sub>-C<sub>36</sub> arylalkanoyl; C<sub>6</sub>-C<sub>36</sub> arylalkenoyl; C<sub>6</sub>-C<sub>36</sub> aryloxyalkanoyl; C<sub>6</sub>-C<sub>36</sub> alkylarylalkanoyl; C<sub>6</sub>-C<sub>36</sub>
- 35 haloarylalkanoyl; C<sub>6</sub>-C<sub>36</sub> aminoarylalkanoyl; C<sub>1</sub>-C<sub>36</sub>

alkylsilanyl; C<sub>1</sub>-C<sub>36</sub> trialkylsilanyl or C<sub>12</sub>-C<sub>28</sub>  
diarylphosphano; or a substituent R', wherein R'  
comprises C<sub>1</sub>-C<sub>36</sub> alkyl; C<sub>1</sub>-C<sub>36</sub> alkenyl; C<sub>1</sub>-C<sub>36</sub> alkynyl; C<sub>1</sub>-  
C<sub>36</sub> haloalkyl; C<sub>1</sub>-C<sub>36</sub> aminoalkyl; halo; amino; C<sub>1</sub>-C<sub>36</sub>  
5 alkylamino; C<sub>6</sub>-C<sub>36</sub> aryl; C<sub>1</sub>-C<sub>36</sub> alkylaryl or C<sub>1</sub>-C<sub>36</sub>  
arylalkyl.

39. A kit according to claim 38, wherein the  
hydrophobic substituent comprises a C<sub>4</sub> to C<sub>7</sub> carbon chain  
10 or ring.

40. A kit according to claim 39, wherein the reactant  
comprises butyric anhydride, pentanoic anhydride,  
hexanoic anhydride or benzoic anhydride.

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41. A kit according to claim 39 or claim 40, wherein  
the proportion of 2'-OH positions bearing the substituent  
is at least 10%.

20 42. A kit according to claim 37, wherein the hydrophobic  
substituent comprises a C<sub>8</sub>-C<sub>12</sub> carbon chain or ring.

43. A kit according to claim 42, wherein the proportion  
of 2'-OH positions bearing the substituent is in the  
25 range 1 to 10%.

44. A kit according to claim 37, wherein the hydrophobic  
substituent comprises a C<sub>12</sub>-C<sub>36</sub> carbon chain or ring.

30 45. A kit according to claim 44, wherein the proportion of  
2'-OH positions bearing the substituent is up to 1%

46. A kit according to any one of claims 37 to 45,  
wherein the separation system comprises a hydrophobic

solid phase for binding the material containing the  
substituent.

47. A kit according to claim 46, wherein the  
5 hydrophobic solid phase comprises hydrophobic particles.

48. A kit according to claim 46 or claim 47, wherein  
the separation system further comprises an elution  
medium for eluting RNA bound to the hydrophobic solid  
10 phase.

49. A kit according to any one of claims 37 to 45,  
wherein the separation system comprises a lyotropic  
salt for aggregating the material containing the  
15 hydrophobic substituent.

50. A kit according to any one of claims 37 to 45,  
wherein the separation system comprises a non-polar  
solvent for forming a hydrophobic liquid phase which  
20 contains the material containing the hydrophobic  
substituent.

51. A preparative device for isolating RNA comprising an  
oligo-or polynucleotide from a sample from a subject,  
25 which device comprises:

(i) a means for extracting the sample from the  
subject;

(ii) a reaction system for modifying RNA in the  
sample to form a modified oligo- or poly-nucleotide in  
30 which a proportion of the 2'-OH positions of the ribose  
rings bear a substituent; and

(iii) a separation system for preparing  
isolated RNA by separating material containing the  
substituent from the sample on the basis of a property  
35 of the substituent.



52. A device according to claim 51, wherein the means for extracting the sample from the subject comprises a syringe needle.

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53. A device according to claim 51 or claim 52, wherein the substituent comprises a solid phase.

54. A device according to claim 53, wherein the solid  
10 phase comprises a membrane, a particle, a bead, a filter, a fibre, a gel, a strip, a matrix, a resin, a capillary or the walls of a vessel.

55. A device according to any of claims 51-54, wherein  
15 the sample comprises biological material.

56. A device according to claim 55, which device further comprises a filter for removing red and/or white blood cells.